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Review

Area Patterning of the Mammalian Cortex

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Here we describe mechanisms regulating area patterning of developing mammalian neocortex, referred to as arealization. Current findings indicate an interplay between intrinsic genetic mechanisms and extrinsic information relayed to cortex by thalamocortical input. Intrinsic mechanisms are based on morphogens and signaling molecules secreted by patterning centers, positioned at the perimeter of dorsal telencephalon, that generate across nascent cortex the graded expression of transcription factors in cortical progenitors. Two major patterning centers are the commissural plate, which expresses *Fgf8* and *Fgf17*, and the cortical hem, which expresses *Bmps* and *Wnts*. Four transcription factors, COUP-TFI, Emx2, Pax6, and Sp8, with graded expression across the embryonic cortical axes, are shown to determine sizes and positions of cortical areas by specifying or repressing area identities within cortical progenitors. They also interact to modify their expression, as well as expression of *Fgf8*. We review these mechanisms of arealization and discuss models and concepts of cortical area patterning.

Introduction

The cerebral cortex is the largest and most complex component of the mammalian brain, and more so than any other brain structure has been affected by evolutionary processes. The result is a tremendous increase in size and complexity across phylogeny, reaching a pinnacle in humans and other primates, cetaceans (e.g., dolphins and whales), and elephants, which have the largest brains among all species (Purves, 1988). The cerebral cortex arises from the dorsal telencephalon, a major subdivision of the differentiated forebrain. The forebrain, positioned at the anterior end of the neural tube, is one of the three major brain vesicles, in addition to the midbrain and hindbrain, that forms early in embryonic development. The dorsolateral forebrain later evaginates to generate the telencephalic vesicles, with the diencephalon (thalamus/hypothalamus) comprising the remainder of the forebrain. The telencephalon is subsequently subdivided into the ventral telencephalon, which gives rise to the striatum and basal ganglia, and the dorsal telencephalon (Rubenstein et al., 1998).

The cerebral cortex itself is divided into regions. The neocortex is the largest region, and is positioned between two other regions of the cerebral cortex, the archicortex (including entorhinal cortex, retrosplenial, subiculum, and hippocampus) and paleocortex (olfactory piriform cortex). In addition, the neocortex accounts for much of the increase in overall brain size and complexity in more advanced species, as well as arguably the most distinct phylogenetic specializations (Krubitzer and Kaas, 2005). Among the many features that distinguish the neocortex from other regions of the cerebral cortex is its laminar patterning characterized by six major, radially organized layers, which themselves are often substratified, each containing a heterogeneous population of neurons that are morphologically, connectionally, and functionally distinct from those of other layers. In its tangential dimension, the neocortex is organized into "areas;" these are functionally unique subdivisions distinguished from one another by differences in cytoarchitecture and chemoarchitecture, input and output connections, and patterns of gene expression (O'Leary and Nakagawa, 2002; Sur and Rubenstein, 2005; Rash and Grove, 2006). In the adult, the transition from one neocortical area to another is typically abrupt, with borders that can be sharply defined by area differences in architecture, and in some instances by the distributions of projection neurons, input projections, or gene expression patterns. These properties determine the functional specializations that characterize and distinguish areas in the adult.

In this article we discuss the mechanisms that operate during development to generate areas of the neocortex, with a focus on genetic mechanisms that operate predominantly within the cortex or around its perimeter to determine the area identities or fates of cortical progenitors and their progeny. Morphogens and signaling molecules expressed in early patterning centers help establish the expression patterns of individual transcription factors (TFs) or combinations of TFs that correlate with morphologic boundaries within the telencephalon (Puelles et al., 2004). These TFs play a prominent role in regionalization of the telencephalon, including establishing and maintaining the identities of the ventral and dorsal telencephalon, and the general characteristics of specific cell types generated within them (Rallu et al., 2002; Schuurmans and Guillemot, 2002). This general genetic theme is reiterated to pattern the neocortex into areas, with an additional contribution from thalamocortical axon (TCA) input that relays sensory input from the periphery to the cortex (Figure 1).

Area patterning is a critical development event, and varies substantially across individuals. For example, the sizes of primary areas in human neocortex vary by 2- to

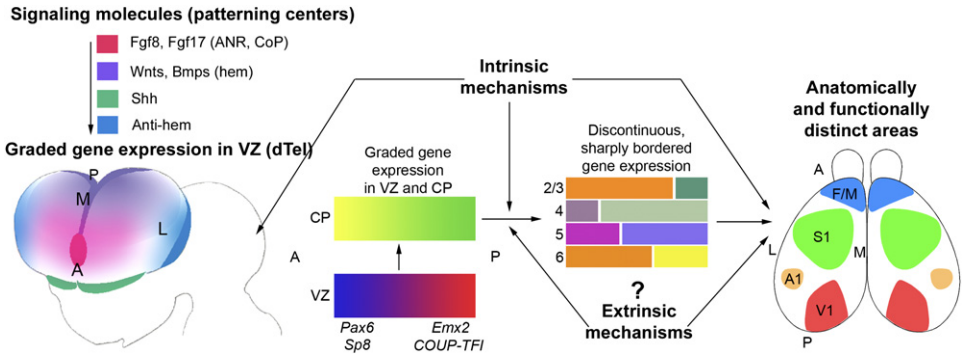

Figure 1. Mechanisms of Specification and Differentiation of Neocortical Areas

The initial, tangential axial gradients of transcription factors (TFs) in the ventricular zone (VZ) are likely established by signaling molecules or morphogens (or both) secreted from localized patterning centers. This figure illustrates four such patterning centers. *Fgf8* and *Fgf17* are secreted from the anterior patterning center, the anterior neural ridge (ANR), which later becomes the commissural plate (CoP). Wnts and Bmps are secreted from the posterior-medial-located cortical hem. Sonic hedgehog (Shh) is secreted from a ventral domain. In addition, a lateral putative patterning center, termed the anti-hem, also might contribute to graded TF expression. In turn, the graded expression of certain TFs, such as *Pax6*, *Emx2*, *COUP-TFI*, and *Sp8*, conveys positional or area identities to cortical progenitors, which are then imparted to their neuronal progeny, which form the cortical plate (CP). The CP also initially exhibits gradients of gene expression that are gradually converted to distinct patterns with sharp borders. Coincident with this process, distinct cortical layers (2–6), and the anatomically and functionally distinct areas seen in the adult (M1, S1, A1, V1), differentiate from the CP. Genes that are differentially expressed across the cortex are often expressed in different patterns in different layers, suggesting that area-specific regulation of such genes is modulated by layer-specific properties, which challenges the definition of area identity. Although the initial establishment of graded gene expression in the embryonic CP is controlled by mechanisms intrinsic to the telencephalon, the much more complex differentiation patterns established postnatally, often both spatially and temporally in parallel with the development of TCA input, suggest that these later events might well be regulated by influences that arise extrinsic to the cortex, such as TCAs. Future studies are required to determine and clarify these mechanisms. The figure is modified from O'Leary and Nakagawa (2002).

3-fold within the normal population (Stensaas et al., 1974; White et al., 1997a, 1997b; Dougherty et al., 2003). In mice, the sizes of primary areas can also vary significantly (Airey et al., 2005). Such variations in area size can have dramatic effects on behavior (Leingartner et al., 2007). For example, genetic manipulations during embryonic development that result in relatively modest decreases or increases in the sizes of somatosensory and motor areas in adults result in significant deficiencies at tactile and motor behaviors. Such findings suggest that areas have an optimal size, influenced by parameters of their neural system, for maximum behavioral performance (Leingartner et al., 2007). They also underscore the importance of mechanisms that operate during development to determine appropriate area sizes (Rakic, 1988; O'Leary, 1989) and thereby influence behavior later in life.

Background

Basic Organization of Neocortex and Thalamic Connections

The neocortex has four "primary" areas; each is the cornerstone of clusters of functionally related areas that include scores of higher-order areas that act as specialized processing centers. Three of the primary areas are sensory: the primary visual (V1), somatosensory (S1), and auditory (A1) areas, which process primary information received from the eye/retina (vision), body (somatosensation), and inner ear/cochlea (audition), respectively. The fourth primary area is motor (M1), which controls voluntary movement of body parts.

The relationship between a primary cortical area and nuclei in dorsal thalamus are critical for both adult function and the developmental differentiation of areas. Dorsal thalamus has four principal thalamic nuclei that functionally and connectionally parallel the four primary cortical areas (Jones, 2007). Each primary cortical area receives TCA inputs from a principal thalamic nucleus that terminate primarily in layer 4, and sends outputs from layer 6 neurons to the same nucleus, thereby generating the reciprocal area-specific/nuclei-specific relationships between cortex and thalamus: the ventrolateral (VL) nucleus with M1, the ventroposterior (VP) nucleus with S1, the dorsal lateral geniculate nucleus (dLGN) with V1, and the ventral part of the medial geniculate nucleus (MGv) with A1. The primary sensory areas receive their major sensory inputs from dorsal thalamic nuclei that define an area's functional modality. The principal sensory thalamic nuclei receive modality-specific sensory information either directly or indirectly from peripheral sense organs or receptors.

The general spatial relationship between the primary areas is largely conserved across mammals, although some animals with unusual or large and atypical peripheral appendages/sense organs (e.g., the platypus' bill or the echolocation system in bats) have modifications on this general geometrical scheme of area patterning (Krubitzer and Kaas, 2005). Historically, areas, or functional fields, of the cortex have also been related to the skull bones that cover them, and in mice the primary areas make up a large proportion of these fields. From anterior (A) to posterior (P), these relationships in mice are as follows: M1 is

covered by the frontal bone and is part of frontal cortex; S1, the parietal bone for the parietal cortex; and V1, the occipital bone for the occipital cortex.

Origins of General Classes of Cortical Neurons

Most neocortical neurons, including all glutamategic neurons and all projection neurons, which are a subset of glutamatergic neurons, are generated in the ventricular zone (VZ) of the dorsal aspect of the lateral ventricle, or at later stages, a second germinal zone, the subventricular zone (SVZ) (Mione et al., 1994; Gorski et al., 2002; Kriegstein and Noctor, 2004). The VZ generates deeper-layer neurons, including subplate (SP) and layer 6 and layer 5 projection neurons; the SVZ is a prominent source of superficial-layer neurons (Kriegstein and Noctor, 2004; Molyneaux et al., 2007). The SVZ is substantially larger in primates than in other mammals, and differences in proliferation in posterior occipital cortex have been reported to contribute to the major increase in the numbers of superficial-layer neurons in V1 compared with adjacent higher-order visual areas (e.g., V2) (Dehay and Kennedy, 2007).

Cortical interneurons, which account for about 20% of all cortical neurons, are GABAergic and also typically express distinct neuropeptides that help define subclasses (Cherubini and Conti, 2001; Krimer and Goldman-Rakic, 2001; Kawaguchi and Kondo, 2002; Butt et al., 2005; Wonders and Anderson, 2006). In mice, they are generated primarily within the medial and caudal ganglionic eminences of ventral telencepahlon and migrate along multiple pathways to reach the cortex (Nery et al., 2002; Ang et al., 2003; Marin and Rubenstein, 2003). Once within the cortex, they migrate along tangentially aligned pathways in the marginal zone (MZ) and intermediate zone (IZ), and eventually turn and migrate radially into the cortical plate (CP), perpendicular to their original tangential path (Nadarajah and Parnavelas, 2002). In primates, a significant number of interneurons are generated within the cortical VZ (Letinic et al., 2002).

A third but proportionally very small general category of cortical neurons are Cajal-Retzius neurons, which populate the MZ (layer 1) and express Reelin, a large secreted protein thought to be required to establish appropriate cortical layering by influencing the radial migration and patterning of cortical neurons (Feng and Walsh, 2001; Ross and Walsh, 2001; Tissir and Goffinet, 2003). Cajal-Retzius neurons are also generated external to the cortical VZ, primarily within the cortical hem but additionally at other sites in the subpallium and septum (Yamazaki et al., 2004; Bielle et al., 2005; Yoshida et al., 2006; Zhao et al., 2006).

Extrinsic Influences on Area Patterning

Although once an intensely debated issue (Rakic, 1988; O'Leary, 1989), it is now widely held that the specification and differentiation of neocortical areas is controlled by an interplay between intrinsic mechanisms, i.e., genetic mechanisms that operate within the cortex, and extrinsic mechanisms such as the sensory periphery and TCA input or information relayed by it (Figure 1). However, until recently, roles for extrinsic mechanisms in controlling area patterning were emphasized for various reasons. One reason is that evidence for intrinsic genetic mechanisms was simply lacking, with the first direct evidence coming only a few years ago with the demonstrations of roles for the TFs *Emx2* (Bishop et al., 2000; Mallamaci et al., 2000) and *Pax6* (Bishop et al., 2000) in specifying the tangential, positional identities of cortical progenitors. However, much positive and compelling evidence for the action of extrinsic mechanisms initially swayed the field, including demonstrations that the cortex is initially a more or less uniform structure, that many area-specific properties differentiate in parallel spatially and temporally to the development of TCA input, and that area patterning and function exhibits considerable plasticity upon modification of sensory periphery or TCA input or performance of heterotopic transplantation (Chenn et al., 1997; O'Leary and Nakagawa, 2002; Sur and Rubenstein, 2005). We will provide a few examples of the action of extrinsic influences in area patterning before discussing roles for intrinsic genetic mechanisms.

Cytoarchitecture and Exuberant Projection Neurons

The properties that distinguish cortical areas gradually emerge during development, with various area-specific features becoming evident at different developmental stages (O'Leary and Koester, 1993; O'Leary et al., 1994; Chenn et al., 1997). The nascent CP, before it acquires its mature functional abilities, lacks most of the anatomically based features that distinguish areas in the adult, even after all CP neurons have been generated and layers begin to differentiate within it. Across its tangential extent, CP cytoarchitecture is uniform other than a smooth anterior-posterior (A-P) and lateral to medial (L-M) decrease in its thickness. Also absent are the restricted, area-specific distributions of distinct types of projection neurons characteristic of the functional specializations of different cortical areas in adults. Instead, cortical projection neurons have widespread distributions early on that include parts of areas, and even entire areas, in which they are not found in the adult; their restricted areal adult distributions come about by the elimination of functionally inappropriate axon segments and branches. This mechanism is used to generate the characteristic areal distributions of layer 5 subcortical projection neurons, as well as callosal and intracortical projecting neurons. Interestingly, though, layer 6 neurons in the primary cortical areas that project to the principal thalamic nuclei appear to exhibit area-specific distributions early on in their development (O'Leary and Koester, 1993; O'Leary et al., 1994; Chenn et al., 1997).

Heterotopic transplant experiments show that area-specific cytoarchitecture and axon/collateral elimination by layer 5 projection neurons is plastic during development. For example, transplants of embryonic occipital cortex, which will differentiate into visual areas, into the S1 barrelfield in parietal cortex develop cytoarchitecture and the patterned expression of markers characteristic of the S1 barrelfield (Schlaggar and O'Leary, 1991). Other

studies show that developing layer 5 neurons transplanted from visual cortex to motor cortex permanently retain their normally transient spinal axon, whereas layer 5 neurons transplanted from motor cortex to visual cortex lose their normally permanent spinal axon and retain their transient axon collateral to the superior colliculus (Stanfield and O'Leary, 1985; O'Leary and Stanfield, 1989). Thus, the projections retained by the transplanted layer 5 neurons are appropriate for the cortical area in which the transplanted neurons develop, not where they were born. The elimination of callosal and intracortical axons is also plastic and is perturbed by a variety of peripheral manipulations of sensory input that alter either patterns of neural activity or absolute levels of activity (O'Leary and Koester, 1993). These and other experimental manipulations reveal tremendous plasticity in the development of the mature areal distributions of projection neurons from initially broad distributions, through mechanisms that are likely to be at least in part independent of the intrinsic specification of area identity.

Area-Specific TCA Input and Potential Roles in Area Patterning

With the possible exception of layer 6 corticothalamic neurons, cortical projection neurons initially exhibit "exuberant" areal distributions far more broad than those in the adult. In contrast to this lack of areal specificity in the early distribution of projection neurons, the area-specific projections of TCAs from the principal sensory thalamic nuclei is evident at the early stages in their development, prior to the emergence of the sharp cytoarchitectonic borders between areas that later become evident (O'Leary et al., 1994). Progress has been made in defining mechanisms of TCA pathfinding, particularly subcortically from dorsal thalamus to the neocortex (Polleux, 2005), but the molecular control of TCA targeting of specific areas remains relatively vague. Similar to the well-defined mechanisms that control development of topographically ordered retinal projections in the visual system (McLaughlin and O'Leary, 2005), area-specific TCA targeting is likely primarily controlled intracortically by graded axon guidance molecules (Dufour et al., 2003) and refined by neural activity (Catalano and Shatz, 1998). SP neurons and their axons have also been implicated in the development of area-specific TCA targeting, but their role and its molecular basis are vague (Allendoerfer and Shatz, 1994; Molnar and Blakemore, 1995).

Since TCAs are the sole source of modality-specific sensory information to the neocortex, the functional specializations of the primary sensory areas are defined in large part by, and dependent upon, TCA input. In addition, the differentiation of many anatomical features that distinguish cortical areas, including architecture and distributions of output projection neurons, depend to a large extent upon TCA input. Consistent with this role, the TCA projection exhibits area specificity throughout its development, and the gradual differentiation of areas within CP parallels the elaboration of the TCA projection within it (Chenn et al., 1997). The plasticity in area-specific architecture and cortical output projections exhibited by heterotopic transplants as described above, as well as the plasticity in architecture and projections induced by peripheral manipulations, demonstrate that the CP exhibits considerable plasticity in the development of area-specific features, and that diverse parts of CP initially have similar potentials to develop features unique to a specific area. Again, TCA input has been implicated as a major influence controlling this plasticity in the differentiation of area-specific features (Figure 1) (O'Leary et al., 1992; O'Leary et al., 1994). In addition, the functional plasticity exhibited by sensory cortical areas revealed by rewiring experiments that alter the modality of sensory input relayed by TCAs to the primary sensory areas further underscores the importance of this input in determining certain area-specific specializations and functions (Sur and Rubenstein, 2005).

The role of TCAs in shaping cortical architecture is not limited to these later events in the differentiating CP. In vitro experiments using mouse tissue suggest that TCAs release a diffusible mitogenic activity that promotes the production of both glia and neurons by explants of the cortical VZ (Dehay and Kennedy, 2007). If a similar mechanism operates in vivo, such an early influence of TCAs on corticogenesis could contribute to the reported areal differences in neuronal production in the SVZ in occipital visual areas (V1 versus V2) in monkey, and could therefore (as described above) contribute to the cytoarchitectural differences between areas that become evident later in development (Lukaszewicz et al., 2005; Dehay and Kennedy, 2007).

Control of Area Identity by Genetic Mechanisms Intrinsic to the Developing Cortex

The initial evidence of roles for intrinsic genetic mechanisms in controlling arealization was indirect and based upon the emergence of differential expression patterns of numerous genes, such as TFs, cell adhesion molecules, and axon guidance receptors and ligands, within cortical progenitors in the VZ or within their progeny in the CP prior to the development of TCA input (Figure 1) (Miyashita-Lin et al., 1999; Nakagawa et al., 1999; Sestan et al., 2001). These expression patterns, most of which are graded across the A-P and M-L cortical axes, have been shown to develop independent of TCA input by analyses of mutant mice with targeted deletion of the TFs *Gbx2* or *Mash-1*, neither of which is expressed in the cortex, but both of which are required for TCAs to reach cortex (Miyashita-Lin et al., 1999; Nakagawa et al., 1999). Subsequently, small differences have been reported in the expression pattern of *ephrin-A5* in *Mash-1* mutants compared with wild-type (Yun et al., 2003), but the major point remains that mechanisms intrinsic to the cortex control the embryonic development of most patterns of differential gene expression.

However, the most dramatic areal changes in gene expression occur postnatally. Many of these late emerging expression patterns align with cortical areas or borders between areas. An example is the transformation of the

expression of the regulatory gene *RORβ* from a graded pattern across the embryonic CP into a disjunctive one mainly limited to layer 4 of the primary sensory areas in postnatal CP. This transformation spatially and temporally closely parallels the postnatal development of patterned TCA input from the principal sensory nuclei to the CP (Nakagawa and O'Leary, 2003). Thus TCA input may well influence or even drive the postnatal differentiation of these more complex gene expression patterns, a process that may be required for proper differentiation of certain area-specific features. If TCA input is found to drive the postnatal patterning of *RORβ* expression and *RORβ* influences the differentiation of areas, one could envision a straightforward, multiple-stage scenario for the proposed interplay between extrinsic and intrinsic mechanisms in arealization. For example, as discussed in a later section, TFs such as Emx2 and COUP-TFI provide an intrinsic genetic framework that specifies the area-specific patterning of TCA input. The influences of TCA input transform the graded expression of *RORβ* to a localized expression in layer 4 of the primary sensory areas. This limited expression of *RORβ* in turn regulates later events in arealization required for the differentiation of the primary sensory areas and the establishment of their unique properties that distinguish them from other areas.

Only in the past few years has direct evidence for the intrinsic genetic control of arealization been reported. Current findings indicate a regulatory hierarchy that begins at the perimeter of the dorsal telencephalon, which includes the nascent neocortex, with morphogens or signaling molecules secreted from patterning centers, which in turn establish within cortical progenitors the differential expression of TFs that determine the areal identity exhibited by their neuronal progeny that form the CP (Figure 1). The two major dorsal telencephalic patterning centers that have been most directly implicated in area patterning are the commissural plate (CoP), which expresses several members of the fibroblast growth factor family (Fgfs), and the cortical hem, which expresses bone morphogenetic proteins (Bmps) and vertebrate orthologs of *Drosophila* wingless (Wnts).

An additional patterning center expresses Sonic hedgehog (Shh) and is located in ventral telencephalon and the hypothalamus of ventral diencephalon (Crossley et al., 2001). Shh secreted by this center is implicated in regional patterning of the forebrain, but only indirectly influences cortical area patterning through its broader functions (Grove et al., 1998; Aoto et al., 2002; Ohkubo et al., 2002; Rallu et al., 2002; Kuschel et al., 2003). Finally, the antihem is a putative patterning center identified by its expression of multiple signaling molecules, such as Tgfα, Neuregulin1, Neuregulin3, Fgf7, and the Wnt antagonist Sfrp2 (Assimacopoulos et al., 2003). The antihem is located in the lateral margin of the cortex near the boundary between dorsal and ventral telencephalon. The cortical hem and antihem have been suggested to cooperate with the anterior neural ridge (ANR)/CoP to establish identities along the A-P and M-L axes of the developing cortex.

The TFs that are expressed by cortical progenitors and have been directly implicated in arealization are presently limited to COUP-TFI, Emx2, Pax6, and Sp8, but certainly include others. In the following sections, we summarize the genetic hierarchy of arealization, focusing on the CoP and the cortical hem, and the TFs that control the sizes and positioning of cortical areas by specifying or repressing areal identities through their expression in cortical progenitors. We first consider evidence of roles for morphogens in area patterning and their regulation, then roles for TFs that directly impart area identities to cortical progenitors.

The Fgf Expression Domain Defines an Anterior Patterning Center

The ANR, which is the anterior junction between neural and nonneural ectoderm, later through morphogenesis becomes the CoP, which is formed by fusion of the neural plate folds at the anterior margin of the forebrain; this structure has been identified as an anterior signaling center for the control of arealization (Figure 1) (Crossley and Martin, 1995; Shimamura and Rubenstein, 1997; Bachler and Neubuser, 2001; Crossley et al., 2001). The ANR/CoP is prominently defined by the discrete expression domains of *Fgf8*, *Fgf17*, and *Fgf18*, 3 of the 22 members of the vertebrate Fgf family that have diverse roles in multiple events during organogenesis (Mason, 2007). Of these, *Fgf8* and to a lesser degree *Fgf17* have been most studied in arealization, and act by locally inducing members of the ETS family of TFs and establishing the gradients of *Emx2* and *COUP-TFI* within cortical progenitors by repressing their expression anteriorly in a dosage-dependent fashion (Garel et al., 2003; Grove and Fukuchi-Shimogori, 2003; Storm et al., 2006; Cholfin and Rubenstein, 2007). Altering levels of *Fgf8* or *Fgf17* has substantial effects on area patterning, presumably through regulation of *Emx2*, *COUP-TFI*, and other TFs expressed by cortical progenitors (Figures 2A and 2B). Anterior overexpression of *Fgf8* by in utero electroporation is sufficient to shift cortical areas posteriorly; in contrast, a similar electroporation of a soluble form of *Fgfr3* that acts as an inhibitor of *Fgf8* shifts areas anteriorly (Fukuchi-Shimogori and Grove, 2001) (Figure 2C). Interestingly, compared with *Fgf8*, *Fgf17* has unique, distinct roles in the patterning of dorsal versus ventral frontal cortical areas (Cholfin and Rubenstein, 2007). Taken together, these results show that the domain of *Fgf8* and *Fgf17* expression in the ANR/CoP functions as an anterior patterning center, and in particular controls frontal/motor cortical area fates.

Fgf8 expression in the ANR/CoP of mice is first detected between E8.0 and E8.5 and is substantially diminished after E13.5 (Crossley and Martin, 1995; Sahara et al., 2007). This expression of *Fgf8* has been suggested to be regulated by several mechanisms that control progressive phases, including initiation and maintenance of expression, as well as restriction of its expression to its normally discrete domain in the ANR/CoP (Figure 3A). Despite the importance of *Fgf8* for cortical development, the

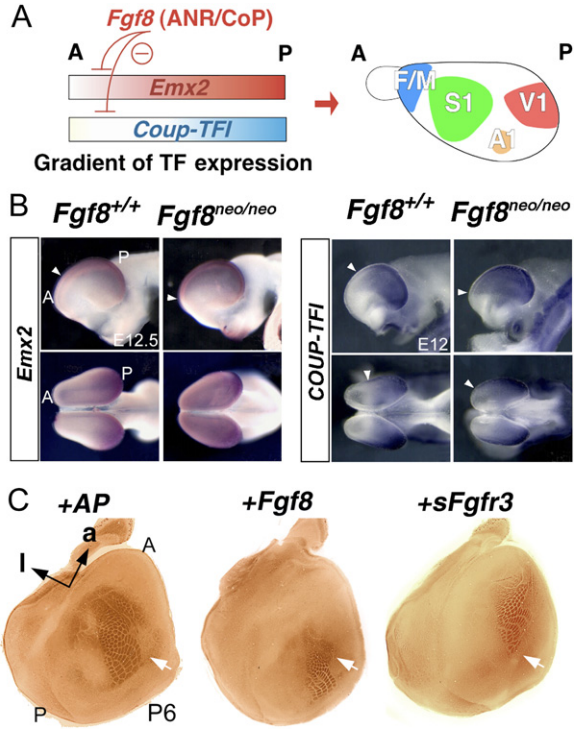

Figure 2. Roles for the CoP and *Fgf8* in Cortical Area Patterning

(A) Schematic views of *Fgf8* action on area patterning. *Fgf8* expressed in ANR/CoP controls the gradients of expression of key TFs in cortical progenitors such as *Emx2* and *Coup-TFI* that have a low anterior and high posterior expression gradient across dorsal telencephalon (dTel). Fgf8 is thought to suppress the expression of both genes in the anterior dTel (red line). The combined actions of graded TFs are thought to establish gene expression with sharp boarders, paralleling the formation of anatomically and structurally defined areas. A, anterior; P, posterior.

(B) *Fgf8* regulates gradients of *Emx2* and *Coup-TFI* expression. Posterior to anterior gradients of expression of *Emx2* and *Coup-TF1* are shifted anteriorly in the dTel of E12/E12.5 *Fgf8* hypomorphic (*Fgf8*$^{neo/neo}$) embryos. Whole-mount in situ hybridization on dissected neural tubes of E12/E12.5 wild-type and *Fgf8*$^{neo/neo}$ embryos was performed with the indicated probes. (Left) The high P-M to low A-L gradient of *Emx2* expression in the dTel is detected in wild-type telencephalic vesicles on lateral and dorsal views. Lateral and dorsal views of an *Fgf8*$^{neo/neo}$ embryo show the anterior shift in the anterior limit of this gradient (compare arrowheads). (Right) The high P-L to low A-M gradient of *Coup-TFI* expression is visible on lateral and dorsal views of wild-type embryos. The anterior limit of high expression (white arrowheads) is shifted anteriorly in *Fgf8*$^{neo/neo}$ embryos. Adapted from Garel et al. (2003); reproduced with permission of the Company of Biologists.

(C) Anterior electroporation of *Fgf8* or *sFGFR3* causes opposite shifts of the S1 barrelfields. Tangential sections through layer 4 of flattened P6 cortices processed for cytochrome oxidase (CO) histochemistry are shown. Patches of high CO activity mark individual barrels in S1. Anterior (a, or A), lateral (l), and posterior (P) are indicated. White arrows mark the midpoint between anterior and posterior poles of the neocortex. From Fukuchi-Shimogori and Grove (2001).

mechanism controlling the initiation of its expression in the ANR remains obscure. Loss of function of the *Hex* gene, expressed in the anterior visceral ectoderm (AVE) (Martinez Barbera et al., 2000), or *Hesx1*, expressed in AVE and anterior neural ectoderm (Dattani et al., 1998; Martinez-Barbera and Beddington, 2001), severely impairs *Fgf8* expression and leads to phenotypes characterized by a truncated forebrain, similar to those seen in *Fgf8* hypomorphic mice, suggesting that these genes are involved in the morphogenesis of the ANR and *Fgf8* expression. Likewise, the *otocephaly* mutant also shows significant reduction of *Fgf8*, although the genes affected in this mutant have not been reported (Zoltewicz et al., 1999). The *Hex* and *Hesx1* homeobox TFs act as repressors for their targets (Martinez-Barbera and Beddington, 2001), suggesting that they may act through intermediaries to influence the initiation of *Fgf8* expression (Figure 3A). Interestingly, *Fgf8* expression in the midbrain persists in *Hex* and *Hesx1* mutant mice, indicating that the expression of *Fgf8* is regulated by distinct mechanisms in its different expression domains, similar in concept to the finding that the LIM homeodomain TF, *Lmx1b*, regulates the initiation of *Fgf8* expression in midbrain but not in the ANR (Andoniadou et al., 2007).

Several lines of evidence indicate that *Fgf8* expression in forebrain is maintained by multiple mechanisms. One mechanism involves the suppression of *Bmp* signaling, which in turn represses *Fgf8* expression (Figure 3A) (Anderson et al., 2002; Ohkubo et al., 2002; Shimogori et al., 2004). The *Bmp* inhibitors *Noggin* and *Chordin* are expressed in the ANR and are required to maintain *Fgf8* expression by inhibiting the activity of *Bmp*s secreted from surrounding nonneural ectoderm (Anderson et al., 2002). Overexpression of *Noggin* is sufficient to induce ectopic *Fgf8* in forebrain at E9.5 but not at later stages (Shimogori et al., 2004), suggesting that Bmp signaling restricts the domain of *Fgf8* expression at E9.5 and earlier, presumably to determine the border between the CoP and the cortical hem/choroid plexus, which are positioned just posterior to the CoP and express *Bmp*s and *Wnt*s (Shimogori et al., 2004). In mice deficient for *Shh*, *Fgf8* expression is initiated but is quickly downregulated by E9.0, indicating that the *Shh* signaling pathway also participates in maintaining *Fgf8* expression (Ohkubo et al., 2002). Although the mechanism is unknown, it is speculated that Shh might suppress Bmp signaling by inducing a *Bmp* inhibitor such as *Gremlin* that may act to derepress *Fgf8* expression (Ohkubo et al., 2002; Panman et al., 2006).

Another mechanism for maintenance of *Fgf8* expression is positive autoinduction by Fgf8 itself (Crossley et al., 1996). In addition, a third mechanism is indicated by recent evidence showing that *Sp8*, a member of the buttonhead family of zinc finger TFs, is a direct transcriptional activator of *Fgf8* expression (Sahara et al., 2007) and is required for its maintenance (Figure 3A) (Sahara et al., 2007; Zembrzycki et al., 2007). These findings are consistent with the early overlap in expression of *Sp8* with the *Fgf8* domain in the CoP (Figure 3B) (Sahara et al., 2007). In knockout mice of *Sp8*, the expression of *Fgf8* is initiated but is prematurely downregulated (Zembrzycki et al., 2007); similarly, overexpression of a dominant-negative form of *Sp8* in the CoP suppresses *Fgf8* expression (Sahara et al., 2007). These data show that *Sp8* is required

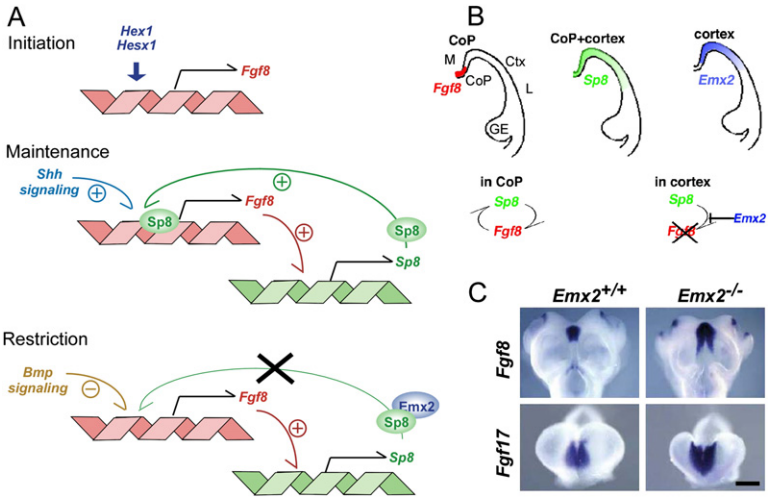

Figure 3. Regulation of *Fgf8* Expression in Forebrain

(A) *Fgf8* expression is regulated in multiple fashions that account for its initiation, maintenance, and restriction to the ANR/CoP. The mechanisms of initiation of *Fgf8* expression are still obscure; however, Hex and Hesx1 are implicated to be upstream of this process since *Fgf8* expression is greatly diminished in ANR in those mutant mice at E8.5, the stage at which *Fgf8* expression is initiated in ANR. Subsequently, *Fgf8* expression is maintained by at least two mechanisms; one is via the Shh pathway, though its signaling to maintain *Fgf8* in the forebrain is still unknown; and the other is via Sp8, a reciprocal inducer of *Fgf8*. Along with the signaling for positive maintenance, *Fgf8* expression is also negatively regulated to restrict its expression domain in ANR/CoP. Bmp signaling suppresses *Fgf8* expression in the early stages of forebrain development by unknown mechanisms. In parallel to Bmp signaling, Emx2 acts as a repressor of *Fgf8* expression by direct binding to Sp8, perturbing its transcriptional activity. Whether transcriptional activity of Emx2 is affected by binding of Sp8 has not been analyzed yet. +, positive interaction; −, negative interaction.

(B) *Emx2* is expressed in cortical progenitors, but not in ANR/CoP, thus; it is likely that Sp8 activity is regulated in a domain-dependent manner. *Sp8* forms a reciprocal induction loop with *Fgf8* in the CoP, and is repressed by Emx2 in the cortex, thereby restricting *Fgf8* expression to the CoP. Modified from Sahara et al. (2007). Ctx, cortex; GE, ganglionic eminence; L, lateral; M, medial.

(C) *Fgf8* and *Fgf17* expression domains extend further laterally, dorsally, and posteriorly in the *Emx2* mutant cortex at E10.5 compared with controls. From Fukuchi-Shimogori and Grove (2003). Scale bar, 400 μm.

for the maintenance of *Fgf8* expression in the ANR/CoP. Further, the reciprocal induction observed between *Sp8* and *Fgf8* (Sahara et al., 2007) might be required for effective *Fgf8* autoinduction.

In addition to being expressed in the ANR/CoP, *Sp8* is also expressed by cortical progenitors in a high-to-low A-M to P-L gradient (Sahara et al., 2007; Zembrzycki et al., 2007), paralleling the presumed diffusion gradient of Fgf8 secreted by the ANR/CoP. The evidence described above and the finding that ectopic expression of *Sp8* is sufficient to induce ectopic *Fgf8* expression suggest that the ability of Sp8 to activate *Fgf8* expression is suppressed in cortex adjacent to the ANR/CoP (Sahara et al., 2007). Independent lines of evidence indicate that this suppressor of *Fgf8* expression in the cortex is the homeodomain TF *Emx2*, which overlaps in expression with *Sp8* but not *Fgf8* (Figure 3B). First, mice with a targeted deletion of *Emx2* have a broader expression domain of *Fgf8*, as well as *Fgf17*, than wild-type does (Figure 3C) (Fukuchi-Shimogori and Grove, 2003); second, in vitro assays show that Emx2 represses the ability of Sp8 to bind regulatory elements of *Fgf8* and induce its expression (Sahara et al., 2007). Thus, Emx2 likely represses *Fgf8* expression in vivo in the cortex and restricts it to the ANR/CoP by suppressing the Sp8 transcriptional activation of *Fgf8*.

Roof Plate/Cortical Hem as a Dorsal/Posterior Signaling Center for Area Patterning

In the spinal cord, the roof plate is a dorsal midline structure that acts as a prominent dorsal-ventral (D-V) patterning center to specify classes of dorsal neurons through its secretion of several signaling molecules, such as Bmps and Wnts (Lee et al., 2000a; Chizhikov and Millen, 2005). In the developing cortex, the roof plate comprises the choroid plexus, which is positioned most dorsally in the cortex, and the cortical hem, which lies at the medial margin of the dorsal telencephalon (Figure 1). Choroid plexus is a neurosecretory epithelium that expresses multiple Bmps. The cortical hem is a neuroepithelium, extending to the dorsal midline from medial cortex, and expresses multiple Bmps and Wnts (Furuta et al., 1997; Grove et al., 1998). On the basis of these characteristics and by analogy to the spinal cord, the telencephalic roof plate is suggested to be a patterning center predominantly involved in specifying more posterior-medial cortex.

It has been suggested that the different telencephalic signaling centers interact during cortical development. For example, ectopic Fgfs and Shh are able to inhibit the expression of Bmps and Wnts (Shimogori et al., 2004; Huang et al., 2007). Reduction of the level of Fgf8 leads to anterior expansion of the *Wnt8b* expression domain (Storm et al., 2006). In *Shh* mutant mice, the loss of Shh leads to increased Bmp signaling (Ohkubo et al., 2002). Mice deficient for *Gli3*, a zinc finger TF that mediates the Shh signaling pathway, show an expansion of the anterior *Fgf8* expression domain and a loss of Bmp and Wnt expression in the roof plate (Grove et al., 1998; Kuschel et al., 2003). Moreover, in embryonic chick telencephalon, focally applied Bmp4 can suppress the expression of *Fgf8* and *Shh*, and the reduction of Bmp signaling by the ectopic expression of Noggin leads to an expansion of the *Fgf8* expression domain (Ohkubo et al., 2002). Thus, these diverse signaling molecules expressed in the three major

telencephalic patterning centers exhibit complex interactions among themselves that likely influence their in vivo patterning functions.

Genetic mechanisms controlling the formation of the cortical roof plate and hem are becoming more clear. Two LIM-homeodomain TFs, Lhx2 and Lhx5, have been shown to be required for the determination and restriction of the roof plate and cortical hem, respectively. Lhx5 is expressed in the cortical hem. The lack of Lhx5 leads to loss of choroid plexus and cortical hem and defective hippocampal formation (Sheng et al., 1997; Zhao et al., 1999). Lhx2 is expressed in the cortical VZ in a high-to-low P-M to A-L gradient, and exhibits an abrupt decline in its expression through a repression by *Bmp2* and *Bmp4* expressed in the roof plate, which excludes Lhx2 expression from the cortical hem (Monuki et al., 2001). The lack of Lhx2 expression alters regional fates and allows the choroid plexus and cortical hem to dramatically expand, whereas in contrast, the neocortex is dramatically reduced in size (Porter et al., 1997; Bulchand et al., 2001; Monuki et al., 2001). These findings suggest that establishing the boundary and fates between the cortical hem and the adjacent cortical VZ requires the action of both Lhx2 and Lhx5 in a complementary fashion.

Although the function of the roof plate in cortical area patterning remains poorly defined, a role is strongly suggested by the pattern and timing of expression of Bmps and Wnts in the cortical hem and their receptors in the cortex. The expression of Bmps in dorsomedial telencephalon is detected as early as E9.5, just after neural tube closure. From E10.5, the expression domain of the Bmps (*Bmp2*, *Bmp4*, *Bmp5*, and *Bmp7*) in dorsal telencephalon is restricted to the dorsal midline (Furuta et al., 1997). Progenitor cells in the dorsal telencephalon exhibit a response to Bmps presumably through their expression of the Bmp receptor *Bmpr-1a*, beginning at E8.0, and *Bmpr-1b*, at E9.0 (Panchision et al., 2001). In developing cortex, the expression of *Wnt3a* is detected in the dorsal midline by E10.5, and the expression of *Wnt3a*, *Wnt5a*, and *Wnt2b* is upregulated in the hem at E11.5 (Grove et al., 1998). Members of the Frizzled (mFz) family of Wnt receptors, mFz-5 and mFz-8, are expressed in the cortical VZ and excluded from the hem early in cortical development, whereas others, mFz-9 and mFz-10, are expressed in the hem. In addition, the Wnt inhibitors Sfrp1 and Sfrp3 are expressed in A-L to P-M gradients within the dorsal telencephalic VZ throughout corticogenesis (Kim et al., 2001).

Studies that alter Bmp and Wnt activities during corticogenesis indicate that they are critical for cortical development. For example, in mice with a cortex-specific conditional knockout of *BmpR1a* to block Bmp activity, the choroid plexus fails to differentiate (Hebert et al., 2002). On the contrary, expression of constitutively active BmpR1a in dorsal telencephalic progenitors results in a dorsalization of the cortex, with the choroid plexus expanding at the expense of the cortex (Panchision et al., 2001). In vitro studies show that Bmps regulate neural apoptosis, proliferation, and gene expression in cortical explants or dissociated cortical progenitors (Furuta et al., 1997; Mabie et al., 1999). Reducing Bmp signaling by electroporating a *Noggin* expression vector into chick telencephalon increases the expression of *Emx2* (Ohkubo et al., 2002), which suggests a role for Bmps in cortical patterning. Wnt-3a signaling is crucial for hippocampal development; it acts locally to regulate the expansion of the hippocampal primordium (Galceran et al., 2000; Lee et al., 2000b). The expression of a constitutively active form of β-*catenin*, a downstream factor in the canonical Wnt signaling pathway, in developing cortex leads to defects in the proliferation and differentiation of neural progenitors (Backman et al., 2005; Chenn and Walsh, 2002). In addition, β-*catenin* is involved in D-V patterning of the telencephalon as a whole. Specifically, inactivation of β-*catenin* in dorsal telencephalon at E8.5 results in downregulation of the dorsal telencephalic markers *Emx1*, *Emx2*, and *Ngn2*, and an upregulation of the ventral telencephalic markers *Gsh2*, *Mash1*, and *Dlx2*. However, deletion of β-catenin at E11.5 has no evident effect on D-V patterning, indicating an early critical period for these patterning influences of β-*catenin/Wnt* signaling (Backman et al., 2005). In addition, Bmps and Wnts have been implicated in the graded expression of *Emx2* in dorsal telencephalon (Theil et al., 2002). In summary, although none of these studies addresses roles for Bmps and Wnts in cortical area patterning directly, this large body of evidence implies such a role; it clearly indicates a significant role for canonical Wnt signaling in the proliferation and differentiation of cortical neurons, and a broader role for Bmps and Wnts in regionalization of the telencephalon (Chenn and Walsh, 2002; Backman et al., 2005).

Roles for the roof plate and cortical hem in cortical development have been further investigated by their genetic ablation. Regulatory elements of the roof plate-specific *Gdf7* gene and the cortical hem-specific *Wnt3a* gene have been used to express the diptheria toxin A chain (DTA) to ablate cells in a tissue-specific fashion; these approaches result in DTA-mediated ablation of the targeted cells around E10 (Monuki et al., 2001; Currle et al., 2005; Yoshida et al., 2006). Ablation of the roof plate causes reduction of Bmp activity in the developing cortex and leads to a reduced cortex size and apparent defects in cortical patterning, characterized by a flattening of the expression gradients of *Emx2* and *Lhx2* (Monuki et al., 2001; Cheng et al., 2006). Surprisingly, though, genetic ablation of the cortical hem has little effect on cortical development (Yoshida et al., 2006). A possible explanation for the absence of patterning defects in the hem-ablated telencephalon is that the cortical hem is involved in cortical patterning prior to its ablation.

Intrinsic Control of Area Identity by Differential Expression of TFs in Cortical Progenitors

As described above, morphogens and signaling molecules secreted by patterning centers have a prominent role in establishing the graded expression of TFs in progenitors in the cortical VZ. These TFs meet the basic

criteria required for candidate genes that specify area identities: regulatory genes differentially expressed across the A-P and M-L cortical axes by progenitors in the VZ, SVZ, or both. These properties suggest that these TFs also function in a differential manner across the cortical axes, which is required to impart area identities, but in addition to differential expression, this property could be achieved by the expression of cofactors or other mechanisms that differentially influence TF function. Functionally, genes that regulate arealization in principle could have a range of effects, from conferring the complete set of properties that comprise the area identity of a cortical neuron, to conferring a subset of these properties, to regulating the expression of axon guidance molecules that control the area-specific targeting of TCAs.

Scores of TFs meet the basic criteria for regulation of area patterning, but to date only four have been reported to function in this manner: *Emx2*, *Pax6*, *COUP-TFI*, and *Sp8* (Figure 6). *Emx2* is a homeodomain TF related to *Drosophila empty spiracles* (*ems*); *Pax6*, a paired box domain TF; *COUP-TFI*, an orphan nuclear receptor; and *Sp8*, a zinc finger TF related to *Drosophila buttonhead*. Within the cortex, the expression of each of these TFs, except *COUP-TFI*, is almost exclusively limited to progenitors in the embryonic VZ; *COUP-TFI* is expressed by both progenitors and CP neurons. *Emx2* is expressed in a high P-M to low A-L gradient (Simeone et al., 1992a, 1992b; Bishop et al., 2000). *Pax6* is expressed in an opposing pattern to *Emx2* along both cortical axes, a low P-M to high A-L gradient (Bishop et al., 2000). *COUP-TFI* has a high P-L to low A-M expression gradient (Liu et al., 2000); thus, it is expressed along the A-P axis in the same gradient as *Emx2*, but in an opposing gradient along the M-L axis. *Sp8* is expressed in a high A-M to low P-L gradient (Sahara et al., 2007); thus, it is expressed along the A-P axis in the same gradient as *Pax6*, but in an opposing gradient along the M-L axis. All but *Sp8* are expressed in progenitors throughout embryonic cortical neurogenesis; *Sp8* is expressed only early in corticogenesis (Sahara et al., 2007). Thus, these four TFs have expression patterns that allow the unique encoding of position along the cortical axes, and therefore in principal area identity.

We want to emphasize that each of these TFs has roles in addition to controlling arealization. For example, Pax6 (Warren et al., 1999) and Emx2 (Heins et al., 2001; but see Shinozaki et al., 2002; Bishop et al., 2003 for opposing in vivo data) have been implicated in control of cell proliferation in cortex, Pax6 regulates D-V regional patterning of the telencephalon and maintains the dorsal telencephalic fate of cortical progenitors (Stoykova et al., 2000; Yun et al., 2001; Kroll and O'Leary, 2005), and as described in a preceding section, Sp8 is a direct transcriptional activator of Fgf8 in the CoP (Sahara et al., 2007). However, here we focus on their roles in arealization.

As summarized in Figure 6, loss-of-function studies have been done in mice for each of these four genes, and gain-of-function studies have been reported for all but *COUP-TFI*. Below we summarize the reported functions for each in arealization. Of these four TFs, roles in area patterning have been most extensively studied for *Emx2*, which we will begin with when describing them.

Emx2

Emx2 expression is highest in progenitors that generate posterior-medial areas of neocortex, such as V1, and lowest in progenitors that generate anterior-lateral areas, such as S1 and motor cortex (Simeone et al., 1992a, 1992b). If Emx2 controls arealization, it should preferentially impart posterior-medial area identities, a prediction confirmed by both loss-of-function and gain-of-function analyses in mice. The initial studies, and the first to show a role for any TF in area patterning, were loss-of-function studies performed on *Emx2* constitutive knockout mice (Bishop et al., 2000; Mallamaci et al., 2000). Subsequent to these first reports, more detailed analyses of *Emx2* knockouts confirmed the findings (Bishop et al., 2002; Muzio et al., 2002; Muzio and Mallamaci, 2003; Li et al., 2006). Surprisingly, though, *Emx1*, a TF very closely related to *Emx2* with a similar graded expression in the VZ, and in addition, in the CP, does not appear to have a significant role in area patterning (Bishop et al., 2002; Muzio and Mallamaci, 2003).

Because *Emx2* knockout mice die at birth, well before cortical areas differentiate, the studies of them were limited to analyses of the patterning of genes differentially expressed in cortex and of reciprocal area-specific connections between dorsal thalamus and cortex. Although the findings matched predictions for Emx2 function, they eventually became controversial because of defects in TCA pathfinding (Lopez-Bendito et al., 2002), a potential region-specific loss of cortical tissue (Muzio et al., 2002) in *Emx2* null mice, and a report concluding that Emx2 acts indirectly in arealization by repressing *Fgf8* expression in the CoP (Fukuchi-Shimogori and Grove, 2003). However, these caveats were circumvented in a subsequent study that showed definite roles for Emx2 in controlling arealization (Hamasaki et al., 2004). This study was based on gain-of-function analyses of *nestin-Emx2* transgenic mice, which use nestin promoter elements to drive elevated levels of *Emx2* expression that are limited to progenitors, and loss-of-function analyses of heterozygous *Emx2* constitutive knockout mice (Hamasaki et al., 2004).

Both cortical size and TCA pathfinding are normal in *nestin-Emx2* mice and $Emx2^{+/-}$ mice, and *Fgf8* expression in *nestin-Emx2* mice is indistinguishable from that in wild-type. In addition, because these mice survive until adulthood, the study allowed the first direct analyses of cortical areas using various techniques that delimit their borders, addressing definitively the influence of Emx2 on the sizes and positioning of cortical areas (Hamasaki et al., 2004). In *nestin-Emx2* mice, the primary sensory and frontal/motor cortical areas have disproportionate changes in their sizes and shifts in position compared with those of wild-type (Figure 4). V1, a posterior-medial area, is significantly increased in size, whereas anterior areas, S1 and frontal/motor, are significantly reduced in

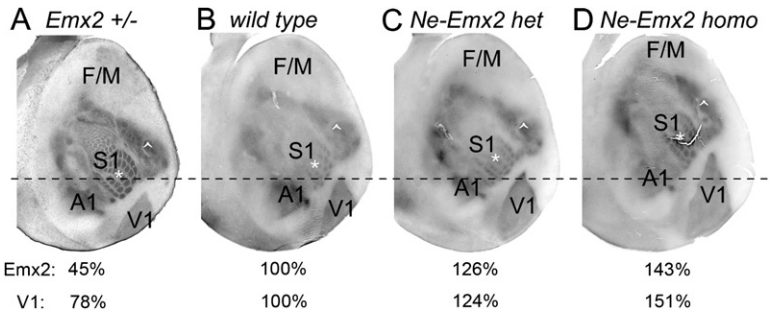

Figure 4. Disproportionate Changes in Sizes of Primary Areas and Shifts in Their Locations Controlled by Emx2 in a Concentration-Dependent Manner

Serotonin immunostaining on tangential sections of flattened cortex of P7 *Emx2* heterozygous knockout (*Emx2*$^{+/-}$, [A]), wild-type (B), heterozygous *nestin-Emx2* transgenic (*Ne-Emx2* het, [C]), and homozygous *nestin-Emx2* transgenic (*Ne-Emx2* homo, [D]) mice. Levels of *Emx2* transcripts increase progressively in the genotypes from (A) to (D), with the lowest level in the *Emx2*$^{+/-}$ mice and the highest levels in the homozygous *nestin-Emx2* transgenic mice. As levels of *Emx2* increase, V1 expands, S1 is reduced in size and shifts anteriorly, and the domain remaining for frontal/motor areas (F/M) is reduced. For each genotype, the level of *Emx2* in embryonic cortex determined by real-time PCR (*Emx2*; Leingartner et al., 2007), and the size of V1 (V1; Hamasaki et al., 2004), relative to that of wild-type (set at 100%), are indicated below each photo. The A-P axis is approximately 8.3 mm. The C3 barrel is marked with an asterisk (*), and the hindpaw representation in S1 is marked with a white ^, to provide references for the anterior shift of the body representation in S1. Figure is modified from Hamasaki et al. (2004).

size; all areas shift anteriorly, and S1 and A1 shift laterally as well. Significant changes are also found in the size and positioning of V1 and A1 in heterozygous *nestin-Emx2* mice intermediate to wild-type and homozygous *nestin-Emx2* mice, whereas anterior areas, S1 and frontal/motor, do not exhibit significant changes in heterozygous *nestin-Emx2* mice. Complementing these gain-of-function studies, *Emx2*$^{+/-}$ mice, which have reduced *Emx2* expression, exhibit a significant reduction in the size of V1 and its anterior border shifts posteriorly, while S1 and frontal/motor areas are increased in size and shifted posteriorly (Hamasaki et al., 2004).

These analyses not only directly delineated cortical areas, but showed that gene markers and TCA input exhibited changes in parallel to the area patterning changes. Thus, Emx2 controls area identities of cortical progenitors and their progeny, as well as positional information that in turn controls the expression of guidance molecules, expressed presumably by SP neurons and possibly CP neurons, that establish area-specific TCA projections. A role for Emx2 in controlling TCA guidance has been shown in a compelling fashion by viral-mediated overexpression of Emx2 in SP and deep CP (Leingartner et al., 2003). TCAs from the dLGN normally project to V1, the cortical area that is generated by progenitors with the highest level of endogenous Emx2. However, in virally infected brains, these TCAs from the dLGN aberrantly turn within the IZ/SP and extend into the CP when they first encounter domains of Emx2 overexpression in parietal (S1) cortex, apparently in response to aberrant positional information specified by the ectopic, high levels of Emx2 that mimic those normally found in V1 (Leingartner et al., 2003).

In summary, genetic manipulations that change the levels of *Emx2* expression in cortical progenitors result in disproportionate changes in the sizes of the primary sensory and motor cortical areas, but have no effect on overall cortical size (Hamasaki et al., 2004). These findings show that Emx2 operates by a concentration-dependent mechanism in cortical progenitors to specify disproportionately the sizes and positioning of the primary cortical areas, and that higher levels of *Emx2* preferentially impart posterior-medial area identities, such as those associated with V1; additionally, as discussed in a later section, these findings led to the "Cooperative Concentration Model" proposed to define the action of TFs that determine area-specific properties in cortical progenitors (Hamasaki et al., 2004).

Genetic rescue studies have validated that *Emx2* controls arealization and that the levels of *Emx2* expression are a critical parameter (Leingartner et al., 2007). These studies were done by crossing the *nestin-Emx2* mice, which have about a 50% increase in *Emx2* expression in cortical progenitors, with *Emx2*$^{+/-}$ mice, which have about a 50% reduction in *Emx2* expression. In the progeny obtained from this cross, both *Emx2* expression in cortical progenitors and the size and positioning of cortical areas are restored to those of wild-type.

COUP-TFI

The initial analysis of a role for COUP-TFI in area patterning made use of constitutive null mice, most of which die within a few days after birth, again limiting analyses (Zhou et al., 2001). These mice exhibit substantial changes in patterns of gene markers, with most markers reported to loose their differential expression along the cortical axes and instead to exhibit broad expression. In addition, TCAs are reported to exhibit aberrant targeting, and layer 4 loses the high density of neurons characteristic of the primary sensory areas. However, these findings and their interpretations are complicated by the robust expression of *COUP-TFI* within forebrain structures, particularly the principal sensory nuclei in dorsal thalamus. Indeed, in *COUP-TFI* constitutive null mice, the majority of TCAs fail to reach the cortex, which compromises an analysis of defects in the targeting of TCAs and changes in layer 4, the principal target layer of TCAs (Zhou et al., 1999). Nonetheless, findings from the *COUP-TFI* constitutive null mice suggest a role for COUP-TFI in arealization.

Recent analyses of conditional *COUP-TFI* knockout mice overcome the complications of the constitutive loss of *COUP-TFI* and demonstrate a dramatic role for COUP-TFI in area patterning (Armentano et al., 2007). The conditional allele of *COUP-TFI* was deleted in these mice by crossing with *Emx1-Cre* mice, resulting in

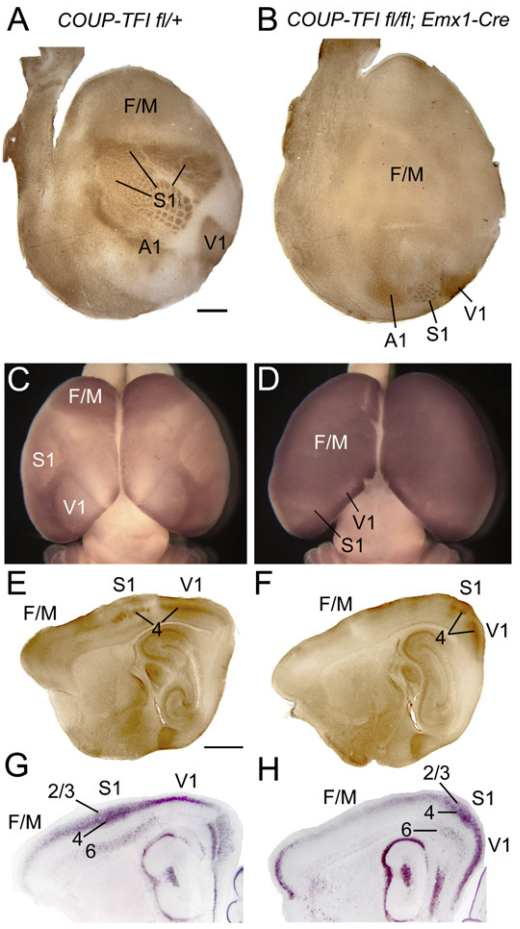

Figure 5. Expansion of the Frontal/Motor Areas and Posterior Compression of Sensory Areas in *COUP-TFI*-Deficient Cortex

(A and B) Serotonin (5-HT) immunostaining on tangential sections through layer 4 of flattened cortices of P7 control (*COUP-TFIfl/+*) and conditional mutant (*fl/fl; Emx1-Cre*) mice. Anterior is to the top, and medial is to the right. (A) Serotonin staining reveals primary sensory areas, including the primary somatosensory (S1), visual (V1), and auditory (A1) areas. V1 is posterior-medial to S1 and A1 is posterior-lateral to S1. Frontal cortex (F) is located anterior to S1. (B) In *COUP-TFI fl/fl; Emx1-Cre* conditional mutant brains, the primary sensory areas are much smaller than in controls and are compressed to ectopic positions at the posterior pole of the cortical hemisphere. The barrelfield of S1 retains its characteristic patterning but is substantially reduced in size and caudally shifted, while a reduced V1 is located medial to, and a reduced A1 lateral to, the miniature S1 barrelfield. (C and D) In situ hybridization for *Cad8* on whole mounts of P7 wild-type (*+/+; Emx1-Cre*) and homozygous conditional mutant (*COUP-TFI fl/fl; Emx1-Cre*) brains uniquely marks the frontal/motor areas (F/M). The F/M massively expands following selective deletion of COUP-TFI from cortex. (E–H) Serotonin (5-HT) immunostaining and MDGA1 in situ hybridization are done on serial sagittal sections of P7 control (*COUP-TFI fl/+*) and conditional mutant (*fl/fl; Emx1-Cre*) cortices. Anterior is to the left, dorsal is to the top. (E and F) Serotonin immunostaining reveals layer 4 of S1 and V1. In the conditional mutant cortex, both S1 and V1 are reduced in size and are ectopically positioned at the posterior pole of the cortical hemisphere (F). (G and H) The S1-specific expression of *MDGA1* in layers 4 and 6 confirms the reduced size and posterior shift of S1, and confirms that these changes occur in parallel across cortical layers in mutant cortex. The majority of the cortex in the conditional mutants, including all of the neocortex anterior to the reduced, caudally shifted primary sensory areas, exhibits levels and patterns of serotonin staining and expression of *MDGA1* that are characteristic of wild-type frontal/motor cortex (F/M). Scale bars, 1 mm. Figure is modified from Armentano et al. (2007).

a selective elimination of *COUP-TFI* expression in the cortex at E10 and later. Cortical deletion of *COUP-TFI* results in a massive expansion of frontal areas, including motor areas, and occupation of most of parietal and occipital cortex, which in wild-type mice are occupied by somatosensory and visual areas, respectively (Figure 5). This frontal area expansion is paralleled by a substantial reduction in the sizes of the three primary sensory areas, which become compressed to the caudal pole of the cortical hemisphere, aligned along its M-L axis. These areal changes are accompanied by changes in area-specific markers, cytoarchitecture, and TCA input to retain area-specific patterns, but they are in parallel to the ectopically positioned areas, and changes in outputs are made to match the expansion of motor areas. Thus, COUP-TFI is required to balance the patterning of neocortex into frontal/motor areas and sensory areas, predominantly by repressing the identities of frontal/motor cortical areas within its expression domain in parietal and occipital cortex, which allows the appropriate specification of the sensory cortical areas.

The continued presence of the primary sensory areas, particularly V1, is likely due to the retained function of *Emx2*. However, the finding that heterozygous conditional knockout mice exhibit an intermediate phenotype indicates that COUP-TFI also has a role in specifying the identities of sensory areas. Although *Emx2* and *COUP-TFI* both exhibit a low to high expression gradient along the A-P axis, they differ substantially in function: Emx2 preferentially specifies posterior area identities in posterior cortex (Hamasaki et al., 2004), whereas COUP-TFI predominantly represses anterior area identities in posterior cortex (Armentano et al., 2007). This difference in function is perhaps best illustrated by the effect of changes in the expression of these TFs on the size of S1 versus V1. Diminishing the expression of *COUP-TFI* has a similar effect on both V1 and S1 (they are both reduced in size), and an opposing effect on frontal/motor areas (i.e., they are increased in size) (Armentano et al., 2007), whereas altering the expression of *Emx2* has opposing effects on V1 and S1 (changes in Emx2 levels that increase V1 size decrease S1 size), and a similar effect on frontal/motor areas and S1 (both are decreased in size) (Hamasaki et al., 2004).

Pax6

Putative roles for Pax6 in area patterning are presently in flux. The initial studies that implicated Pax6 in area patterning depended upon marker analyses of small eye (*sey*) mutant mice, which are deficient for functional Pax6 protein and die at birth (before cortical areas differentiate), have major lamination defects and a cortex reduced by a third, and entirely lack TCA input. Nonetheless, the gene marker analyses implicated Pax6 in specifying anterior area identities associated with motor areas, consistent with its highest expression in progenitors that give rise to anterior areas (Bishop et al., 2000, 2002; Muzio

et al., 2002; Li et al., 2006). However, a recent gain-of-function study of *Pax6* that used a YAC transgenic approach to overexpress *Pax6* several-fold in cortical progenitors reports no changes in area patterning other than a small decrease in S1 size (Manuel et al., 2007).

Although this gain-of-function study fails to reveal a prominent role for Pax6 in area patterning, the dramatic changes in gene marker expression observed in *Pax6* (*sey*) mutants (Bishop et al., 2000, 2002; Li et al., 2006) appear to exceed changes that could be explained solely by the reported preferential loss of rostral cortical tissue in these mutants (Muzio et al., 2002). An appealing explanation for this discrepancy is that another gene or set of genes normally represses the ability of Pax6 to impart frontal/motor area identities in the cortical fields that give rise to sensory areas and could conceivably have a similar action even in the face of *Pax6* overexpression. For example, COUP-TFI, which is expressed robustly by progenitors in parietal and occipital cortex that generate the primary sensory areas, and exhibits a steep decline in expression in frontal cortex (Liu et al., 2000), could have this function if, above a threshold level of its expression, COUP-TFI can repress Pax6's ability to impart frontal/motor area identities to sensory area progenitors.

Sp8

As described in a preceding section, *Sp8* is transiently expressed in the ANR/CoP coincident with the expression domain of *Fgf8* and regulates *Fgf8* expression (Sahara et al., 2007). Two recent studies have reported roles for *Sp8* in arealization. One study employed in utero electroporation of various constructs for gain-of-function and loss-of-function analyses of *Sp8* function in arealization (Sahara et al., 2007), and the other generated a conditional knockout of *Sp8* and crossed it with a *BF1* (*Foxg1*)-*Cre* line to delete *Sp8* from the telencephalon, including *Sp8* expressed in both the ANR/CoP and progenitors in the cortical VZ (Zembrzycki et al., 2007). Because *Sp8* and *Fgf8* reciprocally induce one another, and because *Fgf8* itself has potent effects on arealization by controlling the graded expression of *Emx2*, *COUP-TFI*, and likely other TFs, it is difficult to sort out from these studies the specific role of *Sp8* expression in cortical progenitors in arealization. Nonetheless, analyses of the conditional *Sp8* knockout mice at late embryonic ages show an anterior shift of cortical markers, suggesting that *Sp8* preferentially specifies identities associated with frontal/motor areas (Zembrzycki et al., 2007). Consistent with this finding, an anterior electroporation of a dominant-negative form of *Sp8* in dorsal telencephalon in early embryonic mice (E11) results in an anterior shift of cortical areas, defined postnatally using markers that directly delineate primary areas (Sahara et al., 2007). Additional work is required to address the complexities and specific actions of *Sp8* in arealization and distinguish them from those exerted by Fgf8 or Fgf17.

Interactions between TFs in Controlling Intrinsic Genetic Mechanisms of Area Patterning

Other TFs are likely involved as primary regulators of area patterning and cooperate with the four described above. In addition, the four described here have inductive or repressive effects upon one another that affect their function and their level of expression (Figure 6B). For example, Sp8 is a direct transcriptional activator of *Fgf8*, and Sp8 induction of *Fgf8* is repressed by Emx2 (Sahara et al., 2007), which itself binds Sp8 (Zembrzycki et al., 2007). This provides a mechanism to limit *Fgf8* expression to the CoP, and accounts for the finding of expanded domains of *Fgf8* and *Fgf17* in *Emx2* mutants (Figure 3) (Fukuchi-Shimogori and Grove, 2003). In addition, many of these TFs influence the expression of one another in other ways. For example, Emx2 and COUP-TFI appear to repress *Pax6* (Muzio et al., 2002; Hamasaki et al., 2004; Armentano et al., 2007), and Pax6 appears to repress *Emx2* (Muzio et al., 2002). Further, as described in a preceding section, Fgf8 influences the expression of many of these TFs. In summary, the TFs that control arealization also regulate one another as well as at least a subset of the morphogens (e.g., Fgf8) that establish their graded expression by progenitors in the cortical VZ/SVZ through reciprocal induction or repression loops. This mechanism can modify levels of expression and slopes of expression gradients.

Candidate Targets of TFs That Control Cortical Arealization

Among the critical issues for future studies are defining the targets of TFs that control arealization and how these targets function to generate the many properties that determine the anatomical and functional specializations of an area. A series of recent screens based on RNAs derived from embryonic tissue have been performed to define candidate targets of Emx2 and Pax6 (Arai et al., 2005; Gangemi et al., 2006; Li et al., 2006; Holm et al., 2007). Only one of these screens focused on roles of these TFs in area patterning (Li et al., 2006), but all provide lists of candidate targets for Emx2 or Pax6; these are therefore potentially involved in cortical arealization, as well as functions (including proliferation, neuronal differentiation, migration, axon guidance, and regional patterning of the telencephalon) relevant to other prominent phenotypes exhibited by *Emx2* and *Pax6* (*sey*) mutants. Li et al. (2006) performed a Representational Display Analysis (RDA) comparing *Emx2* null cortex to wild-type, and vice versa, whereas the other three reports are microarray screens that compare neurospheres from *Emx2* null with wild-type cortical progenitors (Gangemi et al., 2006), *Pax6* (*sey*) mutant and wild-type mouse dorsal and ventral telencephalon in multiple combinations (Holm et al., 2007), and *Pax6* (*sey*) mutant rat cortex with wild-type (Arai et al., 2005).

Odz4/*Ten-M4* is an intriguing example of a gene identified uniquely using RDA due to its decreased expression in embryonic *Emx2* null cortex compared with that of wild-type, and subsequently verified using in situ hybridization (Li et al., 2006). *Odz4* is one of four members of a vertebrate gene family (referred to as the *Ten-M* family in mouse) homologous to the *Drosophila* pair-rule patterning gene, *Odd Oz* (*Odz*), which encodes a transmembrane protein involved in segmental patterning in *Drosophila* (Levine et al., 1994) and has structural domains similar

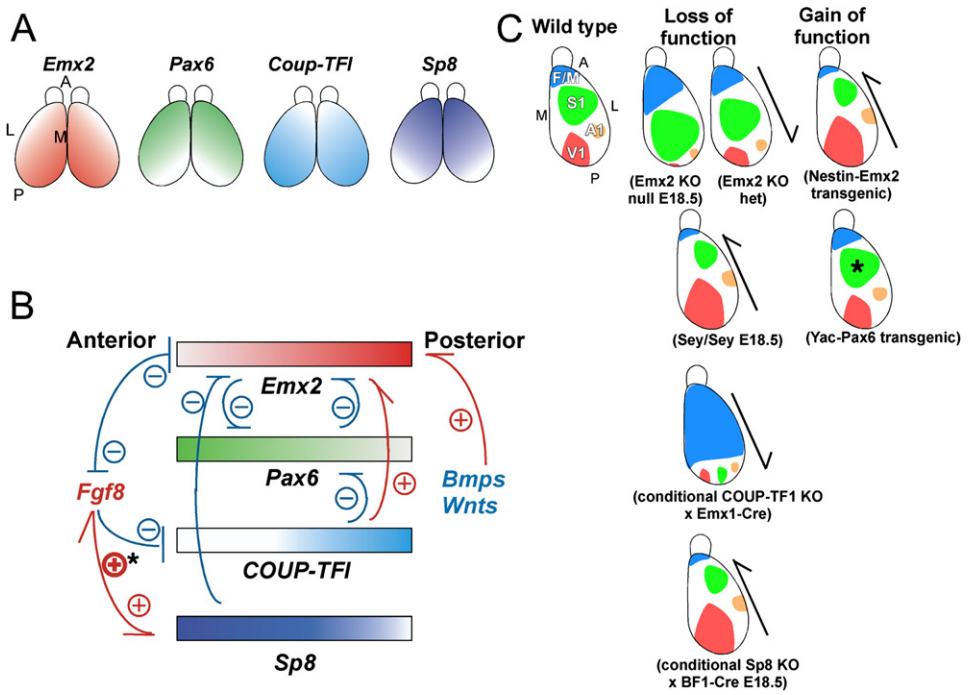

Figure 6. Summary of Intrinsic Genetic Mechanisms of Area Patterning and Mutant Phenotypes

(A) Graded expression of *Emx2*, *Pax6*, *Coup-TFI*, and *Sp8* along anterior-posterior and lateral-medial axes. Key TFs for cortical area patterning show distinct graded expression patterns along anterior-posterior (A, P) and lateral-medial (L, M) axes. *Emx2* is expressed in a high P-M to low A-L gradient. *Pax6* expression pattern is opposite to that of *Emx2*, with a high A-L to low P-M gradient. *Coup-TFI* has a high P-L to low A-M gradient. *Sp8* is expressed in a high A-M to low P-L gradient. While the expression of *Emx2*, *Pax6*, and *Coup-TFI* is sustained in the VZ, *Sp8* expression is quickly downregulated around the onset of cortical neurogenesis.

(B) In the anterior signaling center, *Fgf8* establishes the low anterior-graded expression of the TFs *Emx2* and *COUP-TFI* by repression, and promotes the high anterior gradient of *Sp8* expression. *Fgf8* expression is also regulated positively by direct transcriptional activation by Sp8 through its binding to *Fgf8* regulatory elements, and indirectly by *Emx2*, which represses the ability of Sp8 to directly induce Fgf8, as described in Figure 2. The asterisk marking the activation of *Fgf8* by Sp8 indicates the only interaction that has been shown to be due to direct binding and transcriptional activation (Sahara et al., 2007). Putative posterior signaling molecules *Bmps* and *Wnts*, expressed in the cortical hem, positively regulate the high caudal gradient of *Emx2* expression. Genetic interactions between TFs also participate in the establishment of their graded expression. For example, *Emx2* and *Pax6* mutually suppress each other's expression, *Coup-TFI* suppresses *Pax6* expression and enhances *Emx2* expression, and *Sp8* suppresses *Emx2* expression. Those changes to the expression patterns were identified in the knockout mice; thus, these interactions do not necessarily imply direct control of one TF on another. For instance, *Emx2* suppression by *Sp8* might be due to an enhancement of *Fgf8* expression, which in turn acts negatively on *Emx2* expression. +, positive interaction; −, negative interaction.

(C) Summary of all reports of loss-of-function or gain-of-function mice mutant for TFs that regulate area patterning. Reducing *Emx2* levels in the cortex of the heterozygote mutant mice results in posterior shifts of areas with shrinkage of V1, while overexpression of *Emx2* under the control of the nestin promoter shifts areas anteriorly. The small eye mutant without functional Pax6 shows anterior area shifts as judged by the gene expression patterns of cortical area marker genes, but details of area patterning in postnatal stages of mice lacking *Pax6* has not been reported thus far. Unlike the *Emx2* transgenic mice, YAC transgenic mice of *Pax6* do not show area changes other than a slight, but significant, reduction in the size of S1 (asterisk). Loss of *COUP-TFI* in cortical progenitors transforms the fate of primary sensory areas into frontal/motor areas; thus, *COUP-TFI* represses frontal/motor fates in sensory area domains. The analysis of *Sp8* conditional knockout mice shows anterior shifts of gene markers, a similar phenotype as that seen in *Fgf8* hypomorphic mice. The effects of *Sp8* on area patterning when it is specifically deleted in cortical progenitors have not been reported. See text for details and references.

to tenascin. In embryonic mice, *Odz4* has an expression pattern that parallels the high-to-low P-M to A-L graded expression of *Emx2*, but rather than being expressed in the VZ, Odz4 is expressed in the CP throughout embryonic development. *Odz2* and *Odz3* have similar gradients of expression as *Odz4* in the CP, whereas *Odz1* has an opposing expression gradient (Li et al., 2006). Postnatally, these graded expression patterns refine into more restricted patterns coincident with the differentiation of cortical areas, with *Odz2-4* having patterns that predominantly relate to the posterior-medial positioned visual areas, and *Odz1*, patterns that relate to the more anterior sensorimotor areas. The Odz genes also have distinct laminar expression patterns: *Odz2*, *Odz3*, and *Odz4* have their highest expression in layer 5, and *Odz1*, in layer 4 (Li et al., 2006). Each of the Odz family member exhibits an anterior shift in their cortical expression patterns in *Emx2* mutants and a posterior shift in *Pax6* (*sey*) mutants, consistent with the opposing area patterning functions of Emx2 and Pax6 and potential roles for the Odz genes in arealization as targets of Emx2 and Pax6 (Li et al., 2006).

Defining Area Identities

A defining property of area patterning of the adult cortex is the abrupt transition in anatomical and functional characteristics as one moves from one area to another, marked by sharply defined borders. In addition, many genes expressed in the CP have sharply bordered patterns of expression that often relate to borders of cortical areas, especially as areas themselves emerge and become defined (Figure 1). An important process of arealization during development is translating the graded expression of TFs expressed by cortical progenitors in the VZ/SVZ and early on by their neuronal progeny in the CP into these abrupt, bordered patterns of expression and other properties that relate to and define areas. Little if anything is known about these mechanisms in the developing cortex, but studies in other systems are suggestive. Perhaps the most definitive examples from a mechanistic perspective come from studies of *Drosophila* embryos and their development of the sharply patterned expression of *even-skipped* gene expression, as well as expression of targets of the regulatory protein Dorsal. The graded distribution of Dorsal across the embryo generates, through concentration-dependent differences in binding efficacy to promoter and repressor elements, expression patterns of downstream genes with sharp borders that align with the boundaries of different embryonic tissues and related patterns of gene expression (Rusch and Levine, 1996). The expression of the *even-skipped* gene, limited to multiple, sharp stripes perpendicular to the A-P axis of the embryo, emerges through the combined action of multiple activators and repressors of its transcription; *even-skipped* is expressed where expression of repressors is subthreshold and that of activators is suprathreshold (Rusch and Levine, 1996; Small et al., 1996).

Similar mechanisms appear to operate in the developing vertebrate brain, for example during the differentiation of rhombomeres in the hindbrain (Kiecker and Lumsden, 2005) and establishment of unique progenitor domains in the ventral spinal cord (Jessell, 2000; Shirasaki and Pfaff, 2002). For example, in the spinal cord, Shh secreted by the notocord and floorplate represses or induces the expression of different classes of TFs in the VZ of ventral spinal cord, which initially are expressed in gradients over the D-V axis, similar to the A-P and M-L graded expression of TFs that control area patterning in the cortex. However, in contrast to cortex, in ventral spinal cord the graded expression of these TFs is transformed through mutual repression into sharply bordered expression patterns in the VZ that result in domains of genetically distinct progenitors defined by their expression of distinct subsets of TFs. The distinct domains give rise to different classes of spinal neurons.

Although TFs that control area patterning exhibit mutual repression in cortex (Figure 6B), at no time during neurogenesis are sharply bordered patterns of TFs observed in the cortical VZ; instead, each TF retains a graded pattern across the cortical VZ. Even in the CP, the expression of TFs and other gene families is initially graded before many of them acquire expression patterns with abrupt borders. In the cortex, TFs that control arealization do appear to cooperate to generate area patterning, but these TFs define unique area identities by operating through concentration-dependent mechanisms rather than by establishing distinct domains of progenitors expressing unique sets of TFs. This concept, termed the Cooperative Concentration Model, was developed largely on the basis of studies of the function of Emx2 in arealization (Hamasaki et al., 2004; Leingartner et al., 2007), but where available, the evidence indicates that other TFs involved in arealization work in a similar manner (for example COUP-TFI; Armentano et al., 2007). Overall, the area identities of progenitors in the embryonic cortex are determined by the cooperative interaction of multiple TFs that they express, with a critical difference being the level of expression of each TF.

This discussion leads directly to the major issue of the extent to which areas are genetically distinct in the adult cortex. The available evidence indicates that in terms of gene expression, a neocortical area is not defined by the expression of a gene or set of genes restricted to that area. Instead, neocortical areas are defined by unique patterns of expression of whole sets of genes; each gene that is a member of any given unique set might also be a member of other unique sets that genetically define other areas through their inclusion or exclusion of expression.

Making the term "area identity" even more difficult to define is that each layer has a unique profile of gene expression. Indeed, with a few exceptions, each gene differentially expressed in the neocortex and expressed in more than one layer has different expression patterns in each layer. An example of this feature is the expression profile of *MDGA1*, which encodes a protein that is a cell adhesion molecule of the immunoglobin superfamily (Litwack et al., 2004). *MDGA1* is expressed in layers 2 and 3 throughout the neocortex, and exclusively within S1, is also expressed in layers 4 and 6 (Takeuchi et al., 2007). Thus, *MDGA1* has both layer-specific and area-specific patterns of expression, and although it uniquely defines a single area, S1, its expression is not limited to that area. In conclusion, the term "area identity" in this genetic sense is an amorphous concept—although neurons of an area cooperate to generate that area's unique functional attributes, they are not defined as a population by a genetic tag unique to that area.

Conclusion

The mechanisms that control arealization of the neocortex have received considerable attention over the past two decades. During this period the pendulum has swung from models favoring predominant roles for extrinsically mediated influences, including sensory and TCA input, on controlling arealization to models favoring mechanisms based on intrinsic genetic influences. Several TFs, morphogens, and signaling molecules have been defined to have roles in arealization, and headway is being made in understanding their interactions. Yet the field is nascent

and relatively little is known. Much work needs to be done to better characterize roles in arealization for the TFs presently identified and define additional players near the top of the genetic hierarchy, and to determine their targets and mode of action. In addition, roles of extrinsic mechanisms and TCAs in arealization, which presently are vague and only defined at a phenomenological level, need to be better understood in part because they may well be a significant source of cortical plasticity. These efforts will be critical to understand how a set of TFs expressed at varying levels in cortical progenitors becomes translated into the precisely patterned, specialized anatomical and functional areas that characterize the adult cortex. Further, these efforts are important as the cortex is the source of our individuality.

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